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Effect of season on bovine seminal plasma proteins in Thailand

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ABSTRACT

Although season has been shown to affect bull sperm quality and fertility in some studies, the effect of season on seminal plasma proteins has not been examined. In the present study, seminal plasma proteins were analysed by Fast Protein Liquid Chromatography (FPLC), to separate the phosphorylcholine-binding proteins and heparin-binding proteins from the other proteins. Semen samples were collected from bulls in three seasons: winter, summer and the rainy season. Sperm quality was analysed by flow cytometry and computer assisted sperm analysis, and further aliquots of semen were used to prepare the seminal plasma for FPLC. Meteorological data were available from a location close to the bull station. There were slight differences in sperm kinematics between seasons, but other parameters of sperm quality were not different. Minor differences in the phosphorylcholine-binding proteins were detected according to season, being lower in summer than in winter or in the rainy season, although there were no changes in the heparin-binding proteins. Temperature, humidity and rainfall differed between winter and the rainy season, but no differences were observed between summer and the rainy season except in the temperature humidity index (THI). However, the THI was above the threshold indicative of heat stress in all seasons, which could explain why few seasonal differences in protein composition were detected in this study. Alternatively, the bulls could have been well-adapted to heat stress. In conclusion, there were only slight differences in bull sperm quality and seminal plasma proteins between seasons during this study.

1. Introduction

Several studies have reported that bull sperm quality is affected by season, especially in tropical climates (e.g. Koivisto et al., 2009; Snoj et al., 2013), but also in more temperate regions (Valeanu et al., 2015; Sabés-Alsine et al., 2017). However, other studies have not been able to detect a seasonal effect (Brito et al., 2002; Prastowo et al., 2019). All of these studies have focused mainly on sperm quality while the effect of season on the composition of the fluid portion of semen, i.e. the seminal plasma (SP), has not been examined.

Bovine SP is a complex fluid to support sperm movement and provide transport for the spermatozoa into the female genital tract during mating (Maxwell et al., 2007a; Poiani, 2006; Suarez and Pacey, 2006). It is mostly composed of secretions from the accessory sex glands together with a small volume of fluid from the testis and epididymis (Maxwell et al., 2007; Moura et al., 2007). It contains proteins, minerals, electrolytes, hormones and enzymes (Poiani, 2006) and has a major function in stimulating and supporting spermatozoa, by providing nutrients and a protective environment, and by enhancing sperm motility in the female (Maxwell et al., 2007a; Poiani, 2006).

Some proteins are involved in sperm maturation (Muino-Blanco et al., 2008), whereas others inhibit sperm capacitation and the acrosome reaction (Soubeyrand and Manjunath, 1997; Kaur and Sharma, 2012). Therefore, differences in fertility could be due to variations in SP composition and its effect on both spermatozoa and the inseminated female. Proteins in bovine SP that stimulate capacitation of spermatozoa bind specifically to heparin and choline phospholipids (Miller et al., 1990; Desnoyers and Manjunath, 1992; Therien et al., 1995). The major phosphorylcholine- and heparin-binding protein, PDC-109 (Protein with N-terminus aspartic acid, D, and carboxy terminus Cystine, having 109 amino acids), from bull SP has been isolated and characterized (Calvete et al., 1996; Gasset et al., 1997). This protein binds to choline phospholipids, inducing cholesterol efflux as a prelude to capacitation (Anbazhagan and Swamy, 2005). Since fertilization cannot occur

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without capacitation, PDC-109 is essential for fertility. Binding to sperm membranes is facilitated in a basic, liquid environment (Anbazhagan et al., 2011).

It has been shown previously that fertility-associated proteins are present in bull SP and their respective concentrations can be correlated with fertility (Killian et al., 1993; Cancel et al., 1997; Gerena et al., 1998; Nauc and Manjunath, 2000). Furthermore, it has been shown that PDC-109 has chaperone-like activity, protecting other proteins against thermal stress via suppression of non-specific aggregation and direction into productive protein folding (Sankhala and Swarmy, 2010). Therefore, it would be interesting to investigate whether variations in SP protein composition could contribute to seasonal variations in sperm quality, perhaps through an effect on nutrition.

Analysis of individual proteins may not be particularly helpful in this context since the presence of one protein may compensate for the absence of another. However, the relative proportions of different classes of proteins may be important. One method for separating and quantifying proteins is Fast Protein Liquid Chromatography (FPLC), which separates phosphorylcholine-binding proteins, heparin-binding proteins, and non-heparin-binding proteins (Hansen et al., 2013).

The aim of the present study was to determine if there is a seasonal effect on the distribution of fertility-associated proteins in SP from bulls in Thailand, evaluating the proportions of heparin-binding and phosphorylcholine-binding proteins by FPLC.

2. Methods

2.1. Animals

Six *Bos indicus* bulls of the American Bhraman breed were available for routine semen collection at the North Eastern bull center, Department of Livestock Development (DLD), Khon Kaen, Thailand (Latitude: 16°26' N, Longitude: 102° 49' E). Their body condition score was 3.50 – 3.75, on a scale of 1–5. The age of bulls at the start of semen collection was 8.0 ± 2.60 years (mean 8 ± 2.6 years, range 4 – 11 years). The bulls were housed in an open barn, as described previously (Koonjaenak et al., 2007a) and were fed on grass (*Panicum maximum* and *Brachiaria ruziziensis*), commercial concentrate and minerals supplement. The bulls were kept according to national guidelines on the housing and care of animals.

2.2. Semen collection and preparation of seminal plasma

Semen was collected by artificial vagina approximately once per week according to routine husbandry procedures at the bull center, using the first ejaculation of each bull. The semen was obtained in three seasons: summer (May – June 2014), rainy season (September – October 2015) and winter (January – February 2016). (Note the months stated refer to the time when the samples were collected, not to the duration of the season). After removing an aliquot for sperm quality evaluation, the remainder of the ejaculate was centrifuged at 1800 g for 10 min to pellet the spermatozoa. The supernatant was removed and checked for the presence of spermatozoa; centrifugation was repeated if necessary to remove all the spermatozoa. Aliquots of SP were frozen at -80 °C until required.

2.3. Metereological data

Data on the ambient temperature (°C), humidity (%), and rainfall (mm) at the time were accessed from the North Eastern Meteorological Center (Upper Part), Khon Kaen, Thailand, which is adjacent to the bull center (Koonjaenak et al., 2007a). The meteorological data were used to calculate a Thermal Humidity Index (THI) following a standard formula from the National Research Council (1971), as follows:

$$THI = (1.8 \times T + 32) - ((0.55 - 0.0055 \times RH) \times (1.8 \times T - 26))$$

where T = temperature and RH = relative humidity.

2.4. Separation of seminal plasma proteins by Fast Protein Liquid Chromatography

The SP proteins were separated on column HiPrep 16/10 Heparin FF, 20 ml (GE Healthcare Bio- Sciences AB, Uppsala, Sweden) by FPLC according to Varilova et al. (2006) and Madej et al. (2013) with some modifications. The column was used directly on ÄKTAdesign™ systems (GE Healthcare Bio- Sciences AB, Uppsala, Sweden) with UNICORN™ software for data calculations on line. Samples of SP (0.1 ml = 1.5 mg of protein) were injected through a valve with a 0.5 ml sampling loop. The non-heparin, non-phosphorylcholine-binding proteins, peak 1, 2 and 3 (F1, F2, F3), were eluted with 0.02 M Tris-HCl buffer containing 0.156 M NaCl, pH 7.5. The phosphorylcholine-binding proteins, peak 4 (F4), were eluted with 0.02 M Tris-HCl buffer containing 0.156 M NaCl and 0.05 M phosphorylcholine, pH 7.5. The proteins adsorbed on heparin, peak 5 (F5), were eluted using NaCl gradient (within 10 min) from 0.156 M to 1.5 M in 0.02 M Tris-HCl buffer, pH 7.5. The flow rate was 1 ml/min. Peak height (mAU), peak area (mAU*ml), and percentage of area for each peak in relation to the total area were recorded for each fraction.

2.5. Evaluation of sperm quality

2.5.1. Computer assisted sperm analysis (CASA)

Motility analysis was performed on aliquots (5 μL) using a CEROS II® Version 1.7 (Beverly MA, USA) connected to a microscope (Zeiss, Axiolab A1, Jena, Germany) with a heated stage (38 °C). The following sperm kinematics were evaluated: total motility (MOT; a spermatozoon that moves more than its head length from its original position during the acquisition; progressive motility (a spermatozoon moving with STR > 80 and VAP > 50; PRO, %), slow motility (a spermatozoon moving with VSL < 30 or VAP < 20; SLOW, %), static motility (a spermatozoon moving with VSL < 1 or VAP < 4; STAT, %), Velocity Average Path (VAP, μm/s), Velocity Curved Line (VCL, μm/s), Velocity Straight Line (VSL, μm/s), Amplitude of Lateral Head Displacement (ALH, μm), Beat Cross Frequency (BCF; Hz), Linearity (LIN, VSL/VCL; %), Straightness (STR, VSL/VAP; %) and Wobble (WOB, VAP/VCL; %), Area (μm^2) and Elongation (μm).

2.5.2. Plasma membrane integrity (MI)

Plasma membrane integrity was analysed by flow cytometry after staining with SYBR14 and propidium iodide (PI) (Goodla et al., 2014). Briefly, the samples were diluted with buffer B (patent applied for; J. M. Morrell and H. Rodriguez-Martinez) to a final concentration of 2×10^6 sperm cells/mL (300 μL). The diluted samples were stained with 0.6 μL of 20 μM SYBR14, 3 μL of 24 mM PI (Live-Dead Sperm Viability Kit L-7011; Invitrogen, Eugene, OR) and incubated at 37 °C for 10 min before evaluation using a FC500 flow cytometer (Beckman Coulter, Brea, CA, USA). Excitation was obtained with an argon-ion laser (488 nm). Red fluorescence was detected via a FL3 band-pass filter (610 nm) and green fluorescence was evaluated via fluorescence channel (FL1) band-pass filter (525 nm). In total, 50,000 spermatozoa cell were analysed. After gating to include only spermatozoa, they were classified as living (%) (intact membrane, SYBR14-positive/PI-negative), dead or dying (%) (damaged membrane, SYBR14-negative/PI-positive; or SYBR14-positive/PI-positive, respectively).

2.5.3. Sperm chromatin structure (SCSA)

Sperm chromatin integrity was evaluated as described by Koonjaenak et al. (2007b). Briefly, the sperm samples were mixed 50 μL: 50 μL with Tris-sodium chloride-EDTA (TNE) buffer (0.15 mol/L NaCl, 0.01 mol/L Tris-HCl, 1 mmol/L EDTA, pH 7.4) and snap-frozen in liquid nitrogen vapor before storage at -80 °C. The samples were thawed on ice approximately 20 min before analysis and 10 μL were diluted with 90 μL

of TNE buffer. Partial DNA denaturation in situ was performed by mixing with 0.2 mL of a low pH detergent solution containing 0.17% Triton X-100 (0.15 mol/L NaCl, and 0.08 mol/L HCl; pH 1.2). After 30 s the denatured sperm were stained with 0.6 mL of acridine orange (6 μg/mL in 0.1 mol/L citric acid, 0.2 mol/L Na_2HPO_4, 1 mmol/L EDTA, 0.15 mol/L NaCl; pH 6.0) and were evaluated by flow cytometry within 5 min of acridine orange staining. The standard optical equipment of a FC500 flow cytometer (Beckman Coulter) was used and forward scatter, side scatter, green (FL1, 525 nm band-pass filter) and red (FL3, 610 nm band-pass filter) fluorescence for 10,000 cells was collected. A gate restricting the analysis to spermatozoa was placed in the FSC-SSC dot-plot. The data were analysed using FCS express version 2 (Denovo Software, Thornhill, Ontario, Canada) to calculate the DNA Fragmentation Index (%DFI) (%DFI=red/red+green fluorescence).

2.5.4. Mitochondrial membrane potential

Mitochondrial membrane potential (MMP) of sperm cells was evaluated using the cationic probe 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) (Goodla et al., 2014; Cossarizza et al., 1993). Briefly, the samples were diluted to a final concentration 2.5 × 10^6 sperm cells/mL with buffer B before staining with 1.2 μL of 3 mM JC-1 and incubating at 37 °C for 40 min. After incubation, the stained samples were analysed with a FC500 flow cytometer (Beckman Coulter), using an argon-ion laser (488 nm). Emitted fluorescence was collected using both FL1 (525 nm) and FL2 (575 nm) filters. Green fluorescence was analysed in FL1 and orange in FL2, with compensation between these parameters. Spermatozoa were gated on the FSC-SSC dot-plot and 30,000 cells were classified as having high respiratory activity (%) (Orange fluorescence) or low respiratory activity (%) (Green fluorescence).

2.6. Statistics

A two-way ANOVA was performed using Statplus:mac LE build 6.0.3 (AnalystSoft Inc, Walnut, CA, USA) for each parameter. Factors in the analyses were season and bull, with the different parameters as dependent variables. Tukey HSD correction was used. Differences were considered significant if $p < 0.05$.

3. Results

3.1. Meteorological data

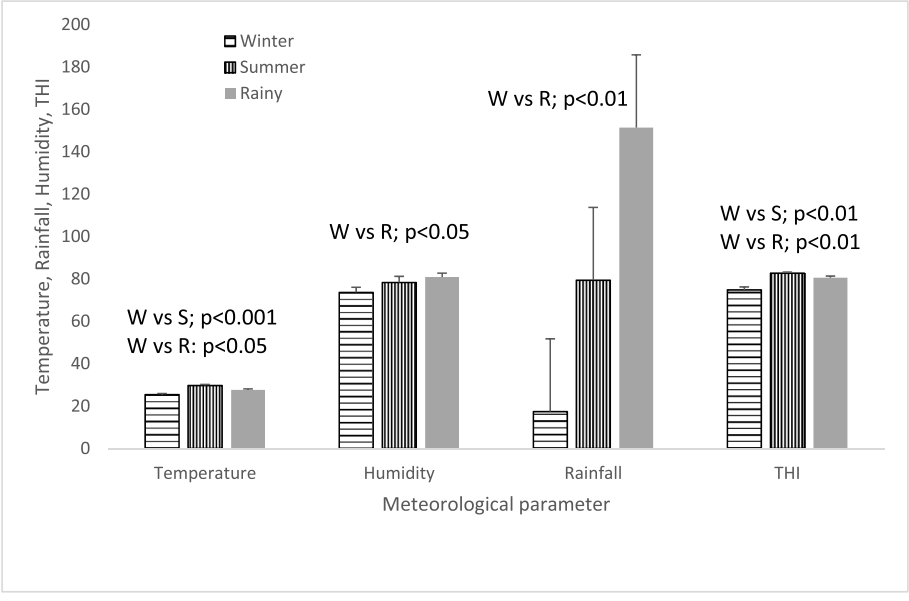
The meteorological data have been reported previously (Nongbua et al., 2020); a summary is provided in Fig. 1.

3.2. Protein separation

Non-heparin-binding proteins were eluted as the three first peaks (F1 + F2 + F3); phosphorylcholine-binding proteins and heparin-binding proteins were eluted as peaks F4 and F5 (Fig. 2). One of the bulls (Bull 3) was considered to be an outlier since the pattern of the FPLC peaks was different to the other bulls and has been excluded.

The peak areas and peak heights of proteins fractions F1, F2, F3, F4 and F5 from the bulls in different seasons are shown in Table 1 and S1, respectively. Table 1a and S1a show the overall means for each peak in each season, while Table 1b and S1b show the mean values for each peak for individual bulls in each season. The peak area of protein fractions 3 and 4 was significantly higher ($P < 0.05$) in ejaculates during the rainy season compared to the ejaculates collected in summer (F3: 414.13 ± 61.32 vs. 265.6 ± 83.67; F4 35.17 ± 18.41 vs. 65.90 ± 20.79). Significant differences in the peak area were not observed in fractions 1, 2 and 5 during all seasons analysed in this study. The peak height of peak 4 was significantly higher ($P < 0.05$) in the rainy season and in winter than in the summer (means ± SD; rainy season 15.01 ± 6.64, winter15.75 ± 5.96, summer 6.62 ± 2.65).

The area of peaks F1-F3 area (Fig. 3) was significantly increased ($P < 0.05$) in the rainy season compared to the summer ($p < 0.05$). The other parameters (peak F1-F3 height; ratio area F4/area F5; height F1+F2+F3+F4/height F5; height F4/height F5; area F1+F2+F3/total area; area F3/total area; area F4/total area; area F5/total area) were not different among seasons for the 5 bulls together ($p > 0.05$; data not shown), although the area of peak 4/total area was greater for Bull 4 (<0.05) in winter than in summer (Table 1b).



Note: multiple scales (Temperature °C; Humidity %; Rainfall mm; THI)

Fig. 1. Meteorological data.
Note: multiple scales (Temperature °C; Humidity %; Rainfall mm; THI).

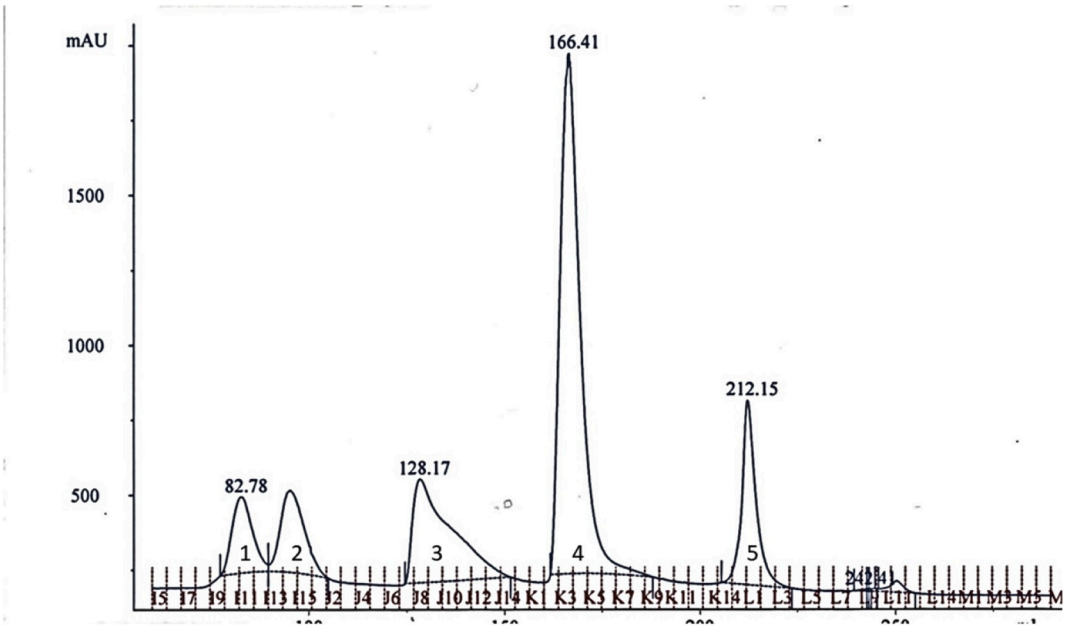


Fig. 2. A representative FPLC profile of bull seminal plasma proteins. Peaks 1, 2 and 3 contain proteins not binding to heparin or phosphorylcholine whereas peak 4 and peak 5 contain phosphorylcholine-binding and heparin-binding proteins respectively.

Table 1a
Peak Area (m Absorbance Units) of protein fractions in different seasons (n = 5 bulls).

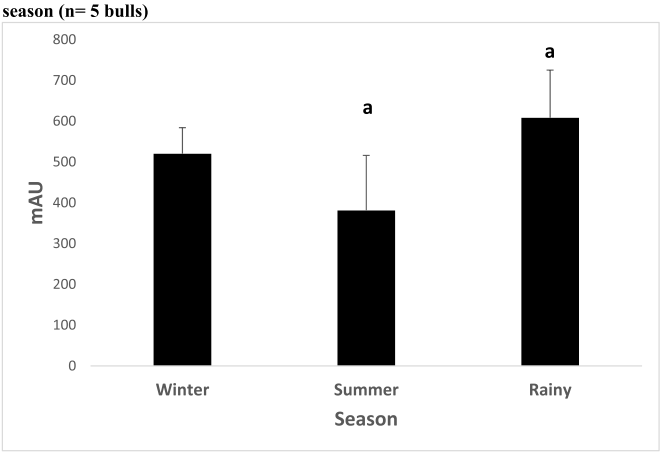
Protein Fractions	Winter	Summer	Rainy
F1	88.60 ± 31.53	53.19 ± 34.53	95.20 ± 31.45
F2	60.15 ± 39.18	62.15 ± 20.89	98.19 ± 49.51
F3	371.11 ± 49.39	265.60 ± 83.67 [a]	414.13 ± 61.32[b]
F4	68.81 ± 17.33 [c]	35.17 ± 18.41 [de]	65.90 ± 20.79 [f]
F5	2.58 ± 2.64	0.26 ± 0.57	2.45 ± 2.82

Note: Overall significant difference among seasons for peaks F1, 3 and 4 ($P < 0.05$). Different superscripts across a row indicate differences between seasons. a,b $p < 0.05$; e,f $p < 0.01$; c,d $p < 0.001$.

Table 1b
Peak area (m Absorbance Units) of protein fractions for individual bulls according to season (n = 5 bulls).

Season	Bull	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
Winter	1	93.05	40.79	430.15	81.89	2.54
	2	99.87	95.24	393.50	74.80	0.00
	4	103.75	2.43	319.45	84.57 [a]	5.88
	5	33.63	68.46	392.71	43.02	0.00
	6	112.69	93.81	319.77	59.79	4.51
Summer	1	94.41	83.54	379.33	61.10	1.28
	2	43.95	81.34	251.13	44.67	0.00
	4	84.44	63.76	320.21	29.63 [ab]	0.00
	5	23.48	37.32	184.45	27.82	0.00
	6	19.65	44.82	192.88 [a]	12.63 [a]	0.00
Rainy	1	81.07	50.48	434.69	65.75	0.00
	2	107.19	68.98	425.66	73.33	2.26
	4	127.76	145.37	382.91	95.95 [b]	6.84
	5	47.65	68.30	331.52	53.31	0.00
	6	112.31	157.83	495.88 [b]	41.15 [b]	3.14

Note: different superscripts indicate differences between seasons within bull. Area of peaks F1-3: significant difference among seasons $p < 0.05$; rainy season > summer, $p < 0.05$.



Note: same letters (a) indicate a significant difference, p<0.05.

Fig. 3. Area of Peak protein fractions 1–3 (m Absorbance Units) according to season (n = 5 bulls).
Note: same letters (a) indicate a significant difference, $p < 0.05$.

3.3. Sperm quality according to season

Sperm kinematics, presented as overall means (Table 2) showed significant differences only for the parameter "area of the sperm head", being higher in winter and summer than in the rainy season ($p < 0.001$). However, when a comparison is made among bulls (Table S2), there were also differences among bulls for VAP, VSL, VCL, Area and motility ($p < 0.05$ for each).

Sperm viability and mitochondrial membrane potential (Table 3) were not different among seasons but showed small differences among bulls ($p < 0.05$).

4. Discussion

The objective of the present study was to compare the distribution of proteins in bull ejaculates collected in different seasons in Thailand using FPLC. There were significant differences only for F4

Table 2
CASA parameters of semen collected in different seasons (means ± SD of 5 bulls).

Season	PRO (%)	MOT (%)	SLOW (%)	ALH (μm)	Area (μm^2)	BCF (Hz)	ELONG (μm)	LIN (%)	STR (%)	VAP (μm/s)	VCL (μm/s)	VSL (μm/s)	WOB (%)
Winter	26 ± 16	72 ± 19	19 ± 3	6.6 ± 1.6	17 ± 1.7 [a]	23 ± 3	0.52 ± 0.05	45 ± 5	76 ± 6	67 ± 16	120 ± 33	51 ± 12	57 ± 3
Summer	25 ± 13	60 ± 25	15 ± 6	6.3 ± 0.8	18 ± 0.5 [b]	24 ± 3	0.47 ± 0.04	46 ± 2	78 ± 2	65 ± 14	114 ± 23	51 ± 12	58 ± 1
Rainy	26 ± 11	76 ± 5	19 ± 7	7.0 ± 1.6	14 ± 0.7[ab]	23 ± 2	0.48 ± 0.03	43 ± 3	76 ± 3	71 ± 19	130 ± 35	54 ± 15	56 ± 2

Same superscripts within a column indicate significant differences between seasons. a, b: $p < 0.001$.
Total motility (MOT), Progressive motility (PRO), Slow motility (SLOW), Static (STAT), Velocity Average Path (VAP), Velocity Curved Line (VCL), Velocity Straight Line (VSL), Amplitude of Lateral Head Displacement (ALH), BCF (beat cross frequency), Linearity (LIN), Straightness (STR), Wobble (WOB), Area of sperm head (AREA), length of sperm head (ELONG).

Table 3a
Mitochondrial membrane potential, membrane integrity and chromatin integrity of semen collected in different seasons (means of 5 bulls).

Season	MMP H (%)	MMP L (%)	LIVING (%)	HG (%)	%DFI
Winter	32	68	27	0.42	5.1
Summer	33	67	24	0.40	7.0
Rainy	30	70	29	0.28	3.9

Note: MMP = mitochondria membrane potential; H, L = high, low; living = membrane intact spermatozoa, HG = High green fluorescence; %DFI = DNA Fragmentation Index.

Table 3b
Mitochondrial membrane potential, membrane integrity and chromatin integrity for individual bulls in different seasons.

SEASON	BULL	HIGH (%)	LOW (%)	LIVING (%)	HG	%DFI (%)
Winter	1	24.06	75.94	16.55	32	4.11
	2	33.48	66.52	25.32	71	8.53
	4	27.55	72.45	25.88	46	0.71
	5	20.87	79.13	19.43	25	9.91
	6	54.31	45.69	46.20	38	2.31
Summer	1	22.59	77.41	9.28	32	11.33
	2	68.11[a]	31.89 [a]	61.38	19	2.91
	4	13.42 [b]	86.58 [b]	6.61	42	9.77
	5	18.89	81.11	12.04	28	9.54
	6	41.14	58.86	42.57	80	1.64
Rainy	1	29.49	70.51	21.87	56	6.64
	2	47.23	52.77	58.05	20	1.58
	4	16.52	83.48	14.79	25	2.2
	5	20.95	79.05	21.67	11	6.68
	6	36.76	63.24	29.93	26	2.19

Note: high, low = high or low mitochondrial membrane potential; living = membrane intact spermatozoa, HG = High green fluorescence, %DFI = DNA fragmentation index.

(phosphorylcholine-binding peak), where the peak height was less in summer than in winter or in the rainy season, and peak area was less in summer than in the rainy season. Among bulls, Bull 4 showed differences in F4 peak height and area. The peak height and peak area indicate how much of a particular component is present, in this case phosphorylcholine-binding proteins. The reason for fewer phosphorylcholine-binding proteins in summer is not known but could be due to factors such as the quality of the feed, or the amount of feed consumed which, in turn, could be associated with water consumption. The temperature and the THI were both highest during summer and might be expected to affect water and feed intake (Collier et al., 2017).

There were small differences among seasons and also among bulls in sperm kinematics (especially for Bull 4), although other parameters of sperm quality were not different among seasons.

One of the problems in comparing our findings with those of others is that the classification of the proteins differs among different authors. Thus, for example, lactoferrin transferase and arginine esterase can be classified as either non-phosphorylcholine binding proteins or as a phosphorylcholine binding proteins, depending on the experimental conditions i.e. which buffer is used, or the order in which different buffers are used. Similarly, lactoferrin and lactoferrin fragments bind both phosphorylcholine and heparin (Mogielnicka-Brzozowska et al., 2017), and would be found in peak 4 in the present experiment, depending on the order in which the different buffers were added.

Thailand has a tropical climate, which might be expected to have an adverse effect on sperm quality. Temperature and humidity were different, and rainfall tended to be different between winter and the rainy season. There were only minor differences in sperm quality among seasons. However, during the period of the study, there were few differences in temperature and humidity between summer and the rainy season, which may be a confounding factor in the interpretation of our results. These results are in agreement with Prastowo et al. (2019) who observed that season did not affect sperm quality in fresh semen from Bali bulls in Indonesia, and also with Brito et al. (2002) who did not find an effect of climate on sperm quality in Brazil.

Factors such as environment, housing, age and breed have been shown to influence sperm quality in some studies (Snoj et al., 2013; Suriyasomboon et al., 2005; Felton Taylor et al., 2020). Although the bulls in our study were kept in an open barn, there was only a slight effect of season on sperm quality. The lack of a difference in climate between summer and rainy season during sampling in the present study could explain why there was not a more obvious difference in sperm quality. Bulls in temperate climates usually show changes in sperm quality for several weeks after changes in temperature and humidity (Malama et al., 2012; Sabés-Alsina et al., 2019); our results indicate that the bulls that are well adapted to their environment show very little effect on sperm quality due to seasonal changes in climate.

In our study, the THI was very similar in summer and the rainy season (83% and 81%, respectively) differing only from winter (75%). However, these THIs are over the threshold at which heat stress is reported to occur, at least for dairy cows e.g. 72–73 (Morton et al., 2007; Schuller et al., 2014). The lack of effect on sperm quality could indicate either that the bulls were already adapted to such conditions, or that they were equally affected by heat stress in all seasons.

In studies with stallion seminal plasma, higher levels of non-heparin, non-phosphorylcholine binding proteins were seen in the non-breeding season than in the breeding season. The authors speculated that these proteins might include cysteine-rich secretory protein 3 (CRISP3), which are known to affect sperm motility (Usuga et al., 2018). The presence of similar proteins could also explain the results seen in the present study, since the area of peak 3 was highest in the rainy season when sperm velocity was also highest. However, this is only speculation at this time; precise identification of the proteins present in each peak was beyond the scope of the present study.

5. Conclusion

Some minor differences in bovine SP protein composition from bulls

in Thailand were detected by FPLC according to season, mainly in the phosphorylcholine-binding proteins. These changes could contribute to seasonal variations in sperm quality, such as in sperm kinematics. However, the differences in temperature and humidity between summer and the rainy season were not marked in the year in which sampling occurred for this study, which could be a confounding factor contributing to the lack of a pronounced seasonal effect in this study. It would be interesting to expand the study to include samples from more bulls and other years.

CRediT authorship contribution statement

I. Lima Verde: Methodology, Investigation, Data curation, Writing - original draft, Visualization. **T. Nongbua:** Conceptualization, Methodology, Investigation, Data curation, Visualization, Writing - review & editing. **S. Karkehabadi:** Methodology, Validation, Resources, Supervision, Writing - review & editing. **A. Johannisson:** Data curation, Formal analysis, Supervision, Writing - review & editing. **J.M. Morrell:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2020.102576.

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